Short Term and Long-Term Effects of Adolescent Traumatic Brain Injury on the Maturation of Executive Functions and Modulation by Sex and Social Stress

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Abstract

It is imperative to understand traumatic brain injuries due to the long-lasting effects of inadequate executive function development and the large spike in TBIs encountered today. Traumatic brain injuries experienced in adolescence may lead to severe behavioral and cognitive alterations that inevitably lead to a poorer quality of life in adulthood. Currently, there is no FDA approved treatment method to TBIs, thus understanding the mechanisms by which executive functions are affected by such injuries can provide valuable insight on how to help and treat individuals who have experienced traumatic brain injuries.

We tested if traumatic brain injuries during the adolescent period affect executive function maturation through the compromise of developmental changes regarding the GABAergic System found in the Prefrontal Cortex. Along with testing for the mild traumatic brain injury (TBI), we also explored and observed the effects of social isolation following the mild TBI. We used a mouse model for this research project. Male and female mice were housed in same sex groups until they reached early adolescence. Once they reached adolescence, half of the mice experienced a mild TBI while the other half received a SHAM surgery. Following the surgery procedure, the mice were then further divided into group-housing or single housing cages to simulate social isolation. To test for long-term effects, the mice remained undisturbed until adulthood at which they underwent various behavioral assays (Puzzle Box, Open Field, and TORM Test) on PD 67 to test their executive functions. In order to test for the short-term effects associated with a TBI, the mice underwent the same behavioral assays described above, but they were not allowed to reach adulthood – they were tested for the same behavioral assays, except they started their behavioral testing on PD 40. After the completion of behavioral testing, Western Blot was run on the PFC to test for the concentrations of Myelin Basic Protein (MBP)

and Parvalbumin (PV). It was found that TBI single housed males, in the short term, showed an increase in latency to enter the goal box in the problem-solving component of the puzzle box test. There were no significant effects found in any of the groups from the TORM test. There was a significant effect of PV expression found in the male SHAM groups, but there was no effect in PV signaling for the females. We found an increase in MBP expression in SHAM males compared to TBI males in the short and long term and we found that TBI females had significantly higher MBP signals compared to female SHAM group. There also seems to be a long-term effect of social isolation in both males and females.

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Introduction

The development and maturation of executive functions are crucial for the fostering of learning and problem solving, after the successful development of these skills, adolescents will be well equipped to carefully plan, focus attention, and successfully perform multiple tasks later in their life (developingchild.hardvard.edu). One of the downsides to executive functions is due to the fact that they continue to develop and do not reach maturity until early adulthood (Satterthwaite et al., 2013). At first, after experiencing a mild traumatic brain injury, adolescents may face subtle difficulties in their everyday life; but, these subtle difficulties have the potential to impact their future by worsening academic ability, affect future employment, and hinder future relationships (Beauchamp et al., 2011). When non-ideal situations arise that negatively affect the successful development of executive functions, including traumatic brain injuries and social isolation, individuals will suffer greatly as a consequence of their their underdeveloped executive functions.

Millions of individuals across the globe have suffered a traumatic brain injury (TBI) (Galgano et al., 2017) and according to the Center for Disease Control (cdc.gov), during the time period of 2001-2010, there has been an increase in traumatic brain injury-related emergency department visits and hospitalizations (cdc.gov). However, due to the increased awareness, structuralizing management, and significant advancements in technology, the number of deaths related to TBIs has decreased throughout this same time period (Galgano et al., 2017). As a result of the increasing number of traumatic brain injuries and the decrease in related death rates, the population of individuals living with disabilities that arose from the traumatic brain injury is steadily increasing.

Not every individual has an equal chance of experiencing a traumatic brain injury. It was found that the highest rates of traumatic brain injuries tend to be in a very young age group (0-4 y) as well as in adolescents and young adults around the ages of 15 to 24 (Galgano et al., 2017). When you group the slow development of executive functions with the higher chance of experiencing a traumatic brain injury especially found in adolescents, you have a recipe for disaster.

There happens to be a sex difference related to traumatic brain injuries observed in adolescents. According to Biswas, Kabir, and King, females are more susceptible to suffer more from the effects of a traumatic brain injury compared to males (Biswas et al., 2017). Although the sex differences might be due to sex hormonal differences, another possibility to account for these differences includes changes in social behaviors following the traumatic brain injury. Studies that utilized the rodent model found that females are far more likely to show evidence of social behavioral impairments after experiencing a traumatic brain injury (Mychasiuk et al., 2014). Social isolation during the critical development period of executive functions negatively impacts the development of cognitive functions. Social isolation can have a great effect attributed to the deprivation of stimuli critical for the maintenance of neurobiological mechanisms and development (Orben et al., 2020) thus leading to a deficit in executive functions.

These findings suggest that the biological sex as well as the environment following the traumatic brain injury could exacerbate the effects of the traumatic brain injury on the development of executive function found in the prefrontal cortex and impairments in cognitive function.

We hypothesize that a traumatic brain injury during adolescence, will delay the development of executive functions by acting on the developmental changes of the prefrontal GABAergic system, and these effects are exacerbated by sex and social isolation.

Purpose:

Given the increase in traumatic brain injuries found in adolescents throughout the last decade and how vulnerable the PFC is during adolescence, it is necessary to understand how these injuries can affect PFC development and how social isolation and sex can exacerbate these effects. This study will help explain the effects sex, TBI, and social isolation have on executive functions in the short and long term. Our findings will provide medical professionals more valuable information on possible treatment methods to lessen the lasting effect of TBI when the victims reach adulthood.

Materials and Methods:

Animals

A total of 82 male and 82 female C57BI/6 (B6) mice were ordered from Jackson Laboratory (Maine, US) and they arrived at PD 21. Male and female mice were separated into 4 groups of n=6 to 10 respectively in order to assess the cognitive effects of a traumatic brain injury and social isolation. All mice were housed in the colony room with *ad libitum* access to food and water. Throughout the whole duration of the experiment, the colony room was kept on a 12-hour reverse light-dark cycle.

Surgery

To administrate a mild TBI, we used the method previously described by Weil et al., 2016. For the TBI and SHAM surgeries, all mice received isoflurane through inhalation to for anesthetic effects. After the administration of the anesthesia, mice were placed in a stereotaxic frame and a round 2mm impactor (Impact One device, Leica Biosystems, Richmond IL) was placed on the surface of the mouse's exposed skull and on the level of the PFC (+1.7 mm AP, +/- 0.3mm ML). The mice in the TBI groups experienced an impact at 3 mm/s (dwell time 30 ms) to a depth of 1mm while the mice in the SHAM groups received an equivalent amount of anesthesia but did not experience an impact. After the surgeries, the skin was closed with nylon suture and mice were allowed to recover from anesthesia.

Behavioral Testing:

Behavioral testing for the first 8 cohorts began 36 days (PD 67), following the TBI or SHAM surgery. Behavioral testing for the second 8 cohorts began 11 days (PD 40), following the TBI or SHAM surgery. Prior to any behavioral test, mice were handled once a day for one minute each for a total of 3 days.

Temporal Order Recognition Memory (TORM) Test:

The TORM test was used to measure the cognitive abilities and memory in the mice. The protocol we used was taken from Wei et al. 2014. On the first two days before testing, mice were habituated to the arena (Pyrex glass square measuring 40 cm by 40 cm) for 10 minutes. Prior to the habituation to the arena, while still in their cages with access to water and food, mice were placed in the testing room under red light for 1 hour before the beginning of the habituation. On

each habituation day, after the hour of habituation in the testing room, each mouse was placed in the arena and was allowed to freely explore the arena for 10 minutes. After each habitation session, the arena was cleaned with 70% ethanol before placing the next mouse in the arena for habituation. Once both days of arena habituation were completed, testing commenced on day 3.

Following habituation to the testing room under red light for an hour, the first day of testing included two sample phases (S1 and S2) followed by a testing phase (T). All three phases were conducted under dimmed white light. The two items used for this test included a glass bottle filled with water and a test tube filled with coins. For the S1 phase, two identical objects were placed in the arena and the mice were then given 5 minutes to freely explore the S1 objects. Following the conclusion of the S1 phase, the mouse was then placed back into their respective cages for a 15-minute inter-trial interval (ITI). After the ITI, two of the identical objects not previously used in S1 were placed in the arena and the mouse was allowed to explore the S2 objects for 5 minutes. Following the S2 phase, there was a 30-minute ITI before the testing phase.

For the testing phase, one object from the S1 phase and one object from the S2 phase were both placed in the arena. Half of the mice were assigned the tubes for S1 and bottles for S2 and the opposite arrangement was used for the other half. For the testing phase, half of the mice were exposed to the S1 object in the left position with the S2 object in the right position and the other half were exposed to the S2 object in the left position and the S1 object on the right position. The mouse was then placed in the arena and was given 5 minutes to explore the objects.

All of the phases were recorded using a video camera and the total time sniffing for the S1 and S2 objects was recorded. For the testing phase, a discrimination ratio was calculated by using the formula depicted in figure 1 below. The higher the discrimination ratio, the greater the

preferance for exploring the older object. If the mouse has a high discrimination ratio, then the mouse has a stronger temporal order recognition memory.

$$\frac{(\text{Time sniffing S1 object} - \text{Time sniffing S2 object})}{(\text{Time sniffing S1 object} + \text{Time sniffing S2 object})}$$

Figure 1: Formula used to calculate the discrimination ratio (DR) for the test phase.

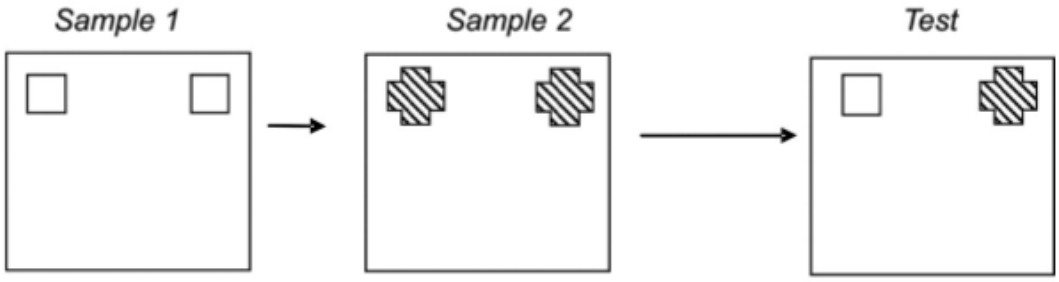


Figure 2: Depiction of the TORM test. Each unique shape represents a different object. The short arrow represents an ITI of 15 minutes and the longer arrow represents an ITI of 30 minutes. Figure taken from (Barker et al., 2007).

Puzzle Box Test:

The puzzle box test is great for evaluating executive functions since it tests for problem solving, learning, short-term memory, and long-term memory. In total, the test phase takes 3 days to complete with a 3-day habituation and food deprivation component prior to the testing phase. During this 3-day habituation and food deprivation period, mice are single housed and handled for one minute daily. On day 1 and 2 of habituation, a petri dish with a ¼ of a froot loop is placed on the floor of the mouse's cage. This is to habituate the mice to the froot loop that will be used during the learning phase of the test. On the third day of habituation, around 20 hours before testing, all food is removed from the cage. This is done to motivate the mice during the learning phase.

The arena used for this test is made of a Plexiglas white box that is divided by a barrier into two compartments. One compartment is a brightly-lit start zone (58 cm by 28 cm) and a smaller covered goal zone (15 cm by 28 cm). These zones are separated by a barrier containing an underpass to the goal box. This underpass is 2 cm deep x 4 cm wide x 4 cm long.

During the 3 days of testing, each day consists of 3 trials. Prior to each day of testing, mice were habituated in the testing room for 1 hour. All of the trials on day 1 are reinforced with a ¼ froot loop in a petri dish located inside the goal zone. For trial 1 (T1), the opening is uncovered and barrier has an open door to the goal zone. The mouse is placed at the far end of the start box and their latency to enter to goal box is recorded. After successfully entering the goal box, each mouse was allowed to remain in the arena for 2 minutes before the beginning of next trial. For trials 2 and 3 (T2 and T3), the barrier has no doorway and the mice are forced to enter the goal box through the small underpass. A froot loop is placed in the goal box like in T1. After the completion of T2 and T3, the arena was wiped down with 70% ethanol before testing a new mouse. Following the conclusion of the first testing day, *Ad libitum* access to food was restored and no food reward was placed in the goal box for the remainder of the trials.

During the second day of testing, trial 4 is identical to T2 and T3 except the fact that there is no froot loop in the goal box for T4. For T5 and T6, the underpass is filled with sawdust (see figure 4) to force the mice to dig their way through in order to reach the goal box. On the third day of testing, T7 is identical to T5 and T6. For T8 and T9, the underpass is obstructed by a cardboard plug (see figure 5) and the mouse is forced to pull the cardboard plug (2g, 2.5cm x 7.5cm x 0.5cm) with their paws and teeth in order to successfully enter the goal zone.

During T5 and T8, when the mouse is exposed to unfamiliar obstacle, problem solving ability is measured while learning and short-term memory is measured with T3, T6, and T9. On trials T4 and T7, that take place 24 hours after the previous trial, long-term memory is tested.

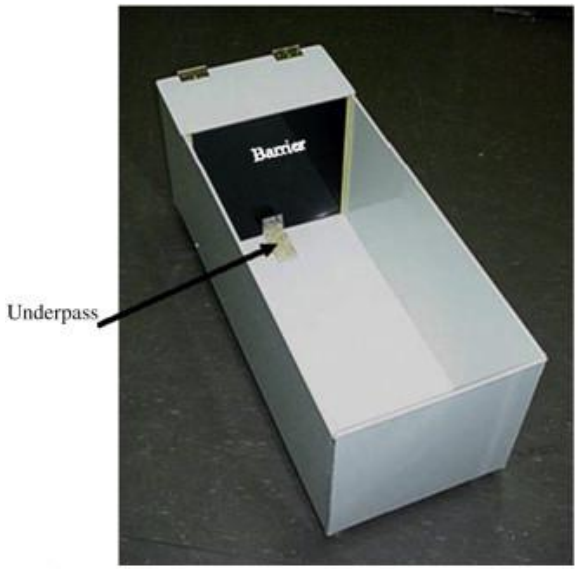


Figure 3: Pictured above is an example of a puzzle box arena. The time for the mice to enter the goal box through the obstacle is measured. Picture taken from (Abdallah et al., 2001).

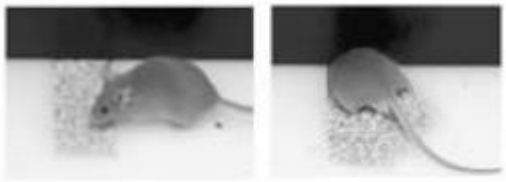


Figure 4: Pictured above are examples of T5, T6, T7 where the mouse has to dig its way through the saw dust to reach the goal box.

Figure 5: Pictured above are examples of T7 and T8 where the mouse has to physically remove the cardboard plug in order to reach the goal box.

Day	**Day 1**	**Day 1**	**Day 1**	**Day 2**	**Day 2**	**Day 2**	**Day 3**	**Day 3**	**Day 3**
Trial	T1	T2	T3	T4	T5	T6	T7	T8	T9
Obstacle	None	Door	Door	Door	Sawdust	Sawdust	Sawdust	Plug	Plug
Tests for:	Training	PS	L/STM	LTM	PS	L/STM	LTM	PS	L/STM

Figure 6: Table above lists each trial done on each day, the obstacle encountered on each trial, and what function the trial tests for. PS = problem solving, L/STM = learning/short-term memory, LTM = long-term memory.

Open Field Test:

The open field test was used in habituation prior to the TORM test. The first open field test (two total habituation open field tests prior to TORM test) was recorded and analyzed using EthoVision. After habituation to the test room for 1 hour, the mouse was placed in a Pyrex glass square measuring 40 cm by 40 cm and were allowed to explore the arena uninterrupted for 10 minutes. Once the open field test was completed, each mouse was carefully placed back in their home cage. EthoVision was then used to measure total time spent around walls and total time spent in the center. The more time the mice spend around the walls is correlated with increased anxiety effects.

Western Blot

Once the mice completed the behavioral testing, their brains were collected at baseline and the PFC was dissected and stored in the -80ºC freezer until day 1 of Western Blot. The Lysate Buffer was prepared by dissolving ¼ Protein Inhibitor Tablet with 2.5 ml T-per. Once the Lysate Buffer was prepared, it was added to each sample determined by the WB lysis calculator (10 mg of tissue + 100ul of Lysate Buffer preparation). The sample was then homogenized until the tissue was completely dissolved and was allowed to sit on ice for 20 minutes. After resting on ice, the samples were centrifuged at 10,000G for 30 minutes at 4ºC and the supernatant was taken. In order to complete the Bradford Protein Concentration Assay, 10ul of the supernatant were taken to calculate and determine how much of each sample would be added to the Western Blot gel. The supernatant was diluted by adding 19ul of ddH_2O per 1ul of sample and the diluted samples were then placed on a microplate next to albumin standards and blanks. Coomassie Plus (Thermo Scientific) was added to each sample and then ChroMate Reader software was used to analyze the samples. Dithiothreitol and Sample Buffer were added to the samples and the samples were then boiled for 5-10 minutes at 95 ºC. Samples were then stored in -20 ºC fridge until running started.

The running buffer was prepared by mixing 3g Tris, 14.4g Glycin, 1g of SDS, and 1L of ddH_2O. The samples were boiled at 95 ºC for 3 minutes before they were then added to the Novex Wedgewell 14% Tris-glycine gel (Thermo Fisher Scientific) along with 4ul of ladder. The running buffer was then placed into the container and the gel was run through gel electrophoresis (Invitrogen Novex XCell SureLock Mini-Cell) at 60V for 30 minutes + 110V for 110 minutes. While the electrophoresis machine was running, the transfer buffer was created by mixing 40ml of 25x TB, 200 ml methanol, and 760 ml of ddH_2O. After the first step of the running was

complete, the transfer membrane was placed with the transfer pads, filter paper, and gel. The filter paper sandwich was then placed in the tank and locked. The transfer was run for 90 minutes at 25V. Once the membrane was transferred, it was washed for 5 minutes in TBS on the shaker (50 RPM) and following the wash with TBS, the membrane was then incubated with the primary antibodies Rb Anti-Myelin Basic Protein (1:1000) ab40390 and Anti-Parvalbumin Protein (1:1000) ab11427 overnight at 4 ºC.

On the next day of the running, the membranes were washed 5 minutes with TBST for a total of 3 times. After washing, the secondary antibody IRDye 800CW Goat anti-rabbi was added to the membrane and remained covered on the shaker (50 RPM) at room temperature for an hour. After incubation with the secondary antibody, membranes were washed with the wash buffer 3 times for 5 minutes each on the shaker (50 RPM) while remaining covered. Membranes were then developed using the LI-COR Biosciences Odyssey CLx Imaging System and analyzed using LI-COR Image Studio.

Statistical Analysis

For data analysis, GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) was used to perform 2-way ANOVA tests with TBI and housing conditions being the independent variables. In the situations were significant effects were found, *post-hoc* Tukey's multiple comparison test was used to compare all of the group means. The results shown in this paper are shown with standard error of the mean.

Results:

TORM Test Short Term Effects

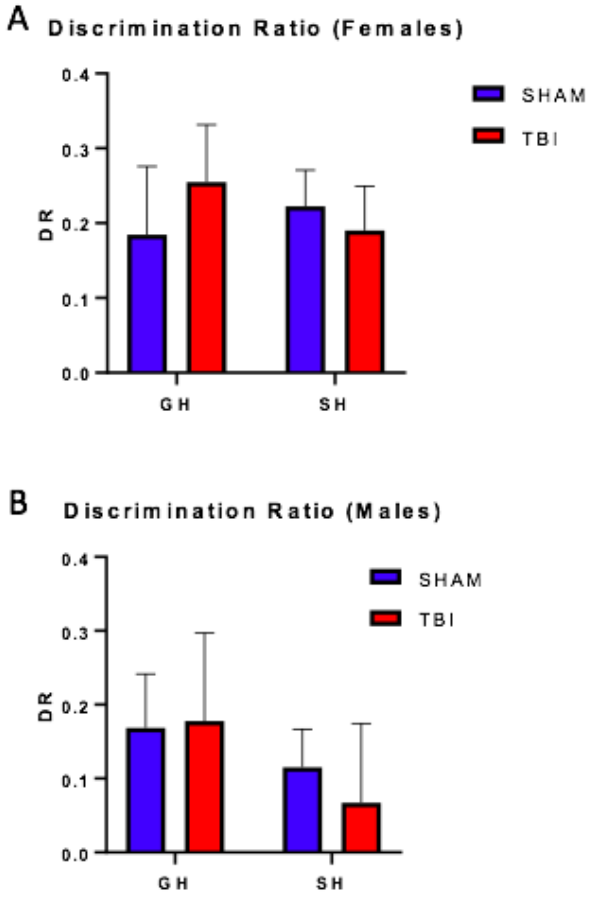


Figure 7: Testing for the short-term effects of TBI and housing, there were no significant TBI or housing effects found in both female discrimination ratio (Figure 7A) and male discrimination ratio (Figure 7B).

Using the formula shown in figure 1, the discrimination ratio (DR) for each mouse was calculated during the testing phase of the TORM test. The higher the DR, the more time the mouse spent sniffing the older object which shows that the temporal order recognition memory is functioning effectively. For females (figure 7A), no significant TBI or housing effect were found in the short term. For males (figure 7B), there were also no significant TBI or housing effects found in the short term.

TORM Test Long Term Effects

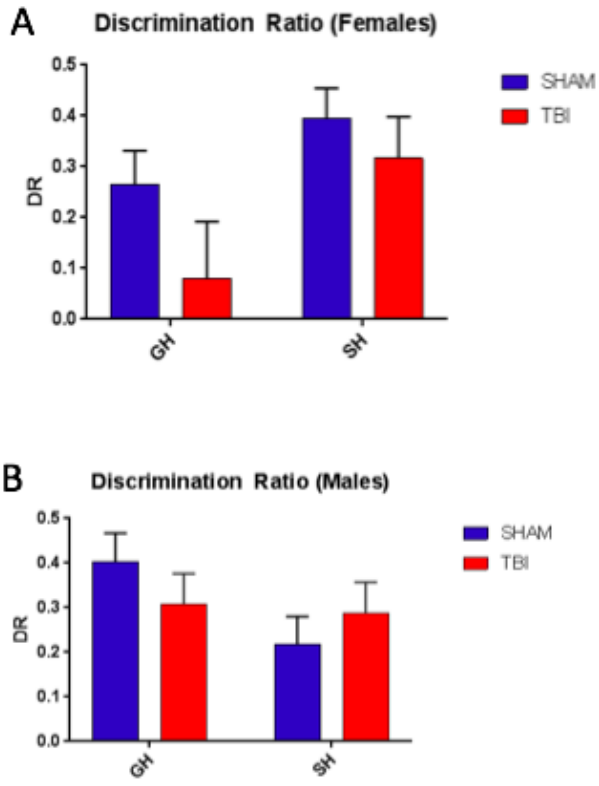


Figure 8: Testing for the long-term effects of TBI and housing, there were no significant TBI or housing effects found in both female discrimination ratio (Figure 8A) and male discrimination ratio (Figure 8B).

After reaching adulthood, all the mice underwent testing for their temporal order recognition memory using the TORM test and their DR was calculated during the testing phase. There were no significant TBI or housing long-term effects found in females (figure 8A). The absence of a significant effect found in discrimination ratio between the female groups tells us there was little to no long-term effect of the mild TBI experienced in adolescence. In figure 8B, no significant effects were found for males across all groups for the long-term effects of housing and TBI signaling no significant long-term effects as a result of a mild TBI in adolescence.

Puzzle Box Short Term Effects (Females)

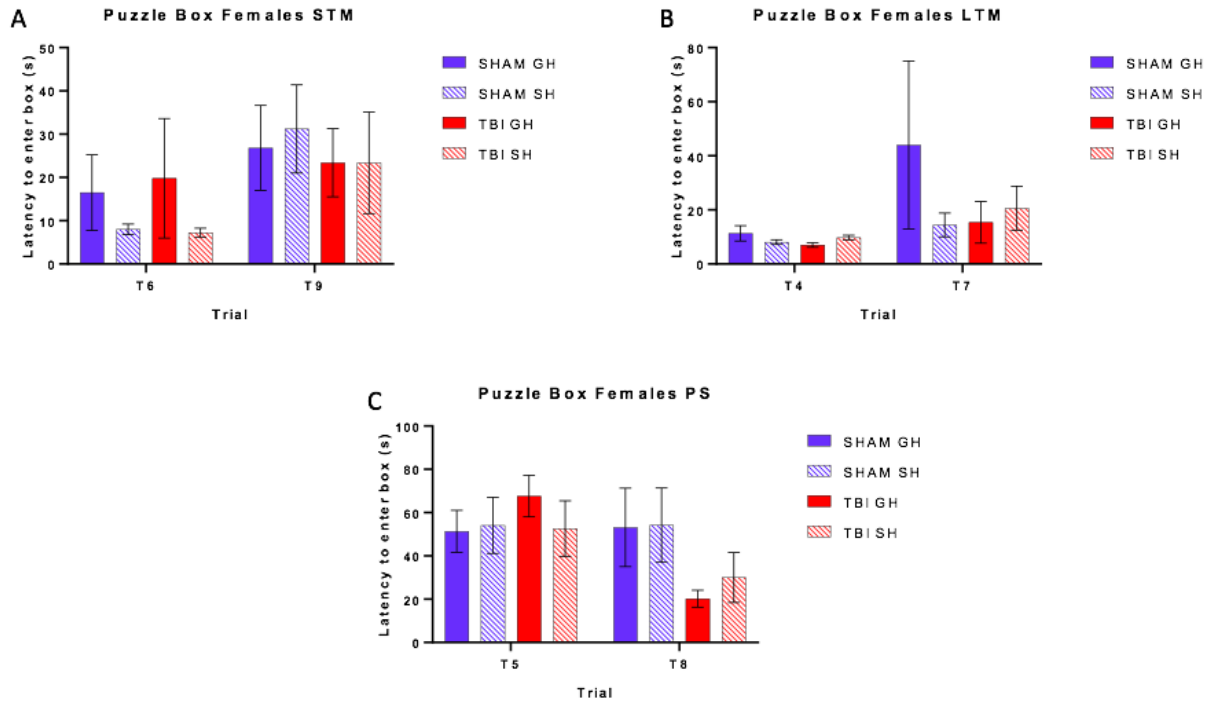


Figure 9: Testing the females for the short-term effects of TBI and housing, there were no significant differences in the latency to enter the goal box throughout all trials. Figure 9A depicts the results for the trials associated with short term memory while Figures 9B and 9C depict the results for trials associated with long-term memory and problem solving respectively.

While still in adolescence, female mice underwent the puzzle box behavioral assay to quantify the latency to enter the goal box while overcoming a variety of obstacles. The puzzle box behavioral assay tests for executive functions such as short-term memory, long-term memory, and problem solving. In the short term, there were no significant effects found across all groups in any of the trials associated with short-term memory, long-term memory, and problem solving.

Puzzle Box Short Term Effects (Males)

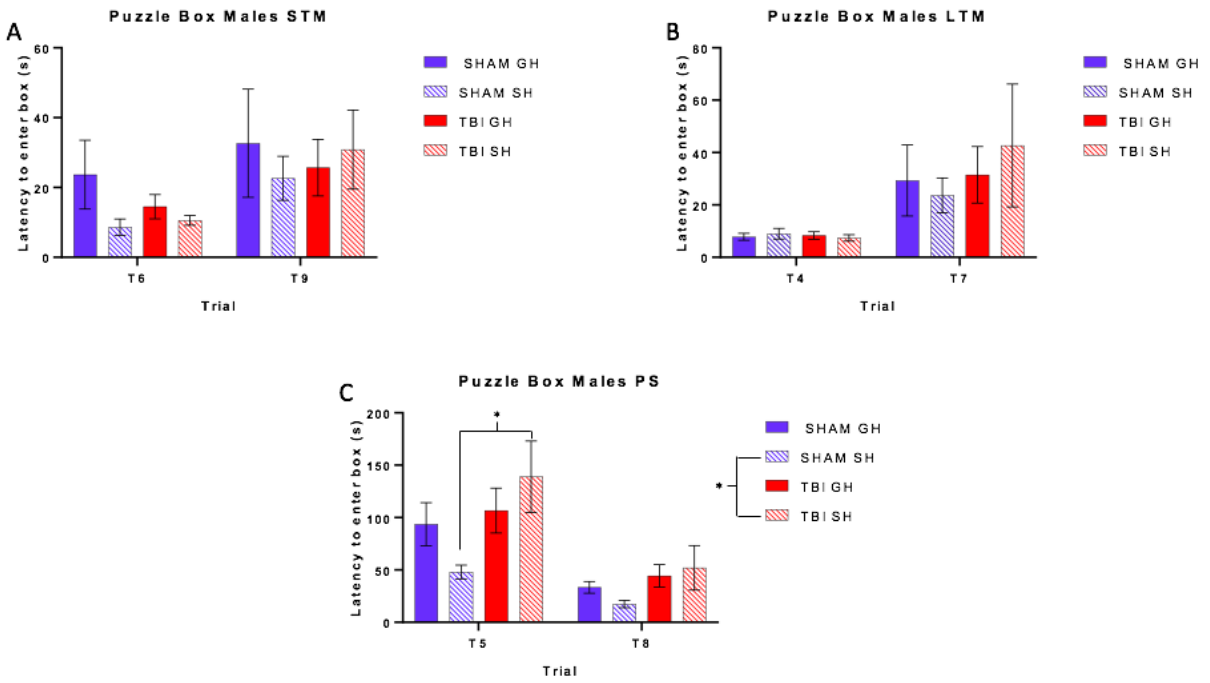


Figure 10: A significant difference between the SHAM SH and TBI SH groups (p=0.0028) was found and pictured in figure 10C. There were no significant differences found in TBI and housing groups in trials that tested for short term memory and long-term memory as depicted by figures 10A and 10B respectively.

As depicted by figure 10C, for the single housed groups, mice who received a TBI were significantly more latent to enter the goal box compared to their SHAM single housed counterpart (p=0.0028). Aside from the SHAM single housed and TBI single housed groups, there were no other significant effects found across all groups for the remainder of the trials. The significant results depicted from figure 10C suggest that there is a TBI effect associated with problem solving skills and those who experience a traumatic brain injury may experience difficulties with executive functions, especially problem solving, shortly following the TBI injury.

Puzzle Box Long Term Effects (Females)

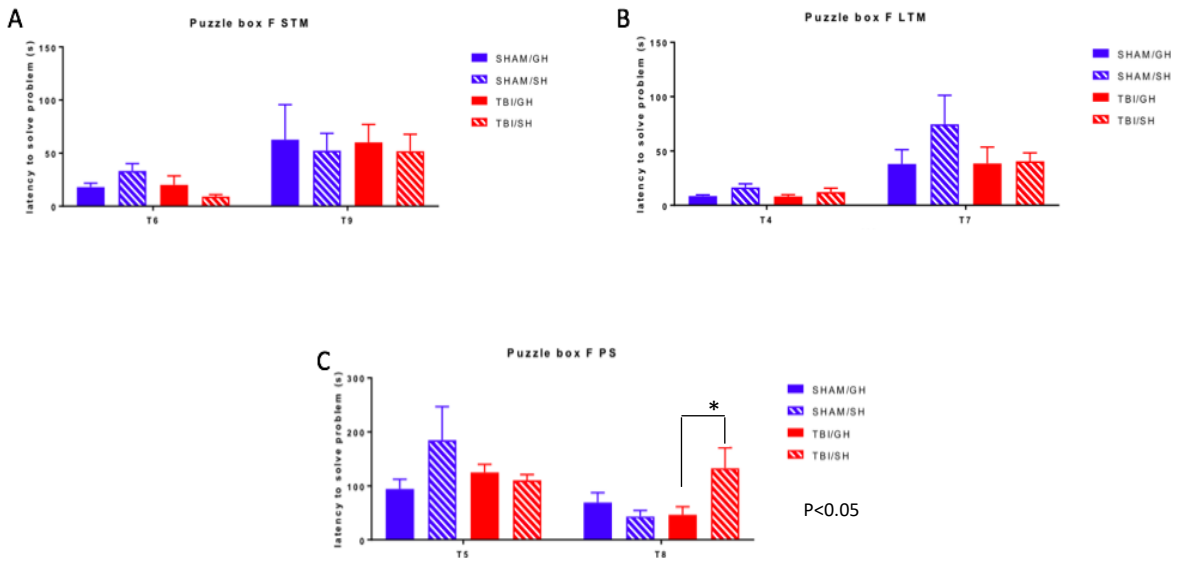


Figure 11: Testing the females, for the long-term effects of TBI and housing, no significant differences in the latency to enter the goal box were found in figures 11A and 11B. However, figure 11C depict the results from trials associated with problem solving and there is a significant housing interaction with a p value of less than 0.05.

When given the ability to reach adulthood, female mice were tested in the puzzle box arena and encountered a plethora of obstacles in order to reach the goal box. As shown in the figures above, there were no significant effects of a TBI effect or a housing effect across the trials associated with short-term and long-term memory. However, taking a look at T8, which tests for problem solving, you can see that the TBI single house group was the most latent to enter the goal box compared to the other groups. This relationship is significant with a p value of less than 0.05. Figure 11C is the only figure that shows female TBI single house mice were the most affected during the testing for problem solving in the long term.

Puzzle Box Long Term Effects (Males)

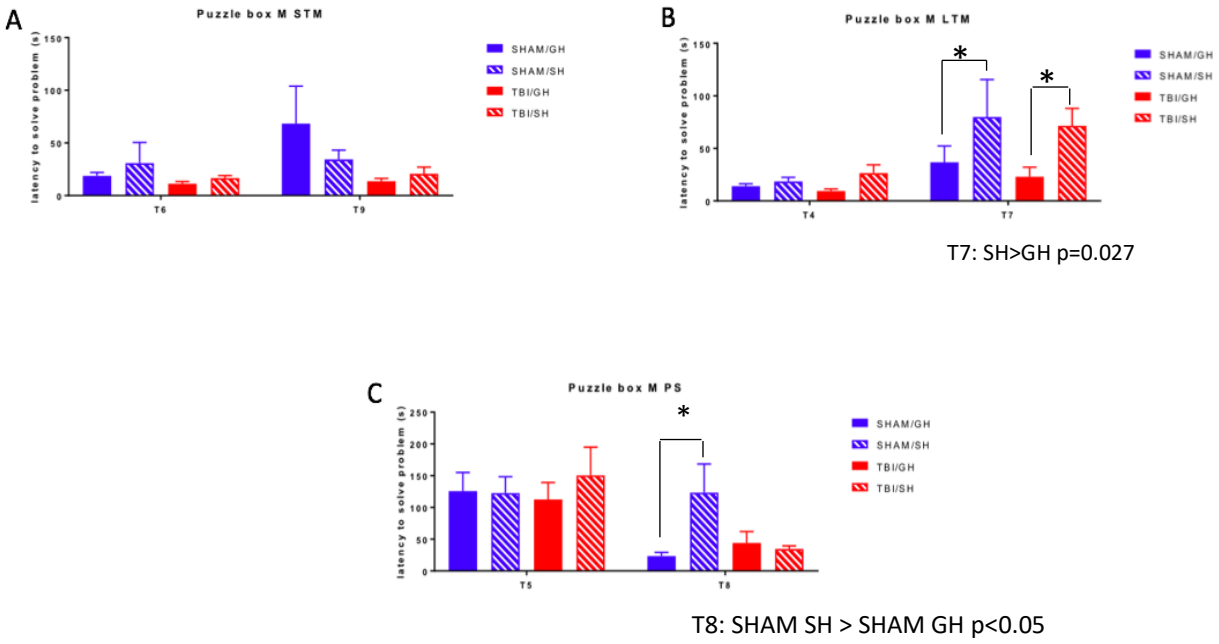


Figure 12: Testing the males, for the long-term effects of TBI and housing, no significant differences in the latency to enter the goal box was found throughout all trials associated with short term memory. In figure 12B, there was a significant interaction with housing conditions with a p value of 0.027. Figure 12C also depicts a housing effect for T8 with a p value of less than 0.05.

No significant interactions between TBI and housing were found in the adulthood testing of the puzzle box for trials associated with short-term memory. In figure 12B, you can see a significant effect in housing displaying that single housed males were significantly more latent to enter the goal box. For the long-term memory component of the puzzle box test, the single housed mice were found to be significantly more latent to enter the goal box compared to the group housed mice. The p value associated with this interaction was found to be less than 0.05. In figure 12C, there is a significant effect of housing showing that single housed SHAM males were more latent compared to the group housed SHAM males and the p value associated with this interaction is less than 0.05.

Open Field Test Short Term Effects

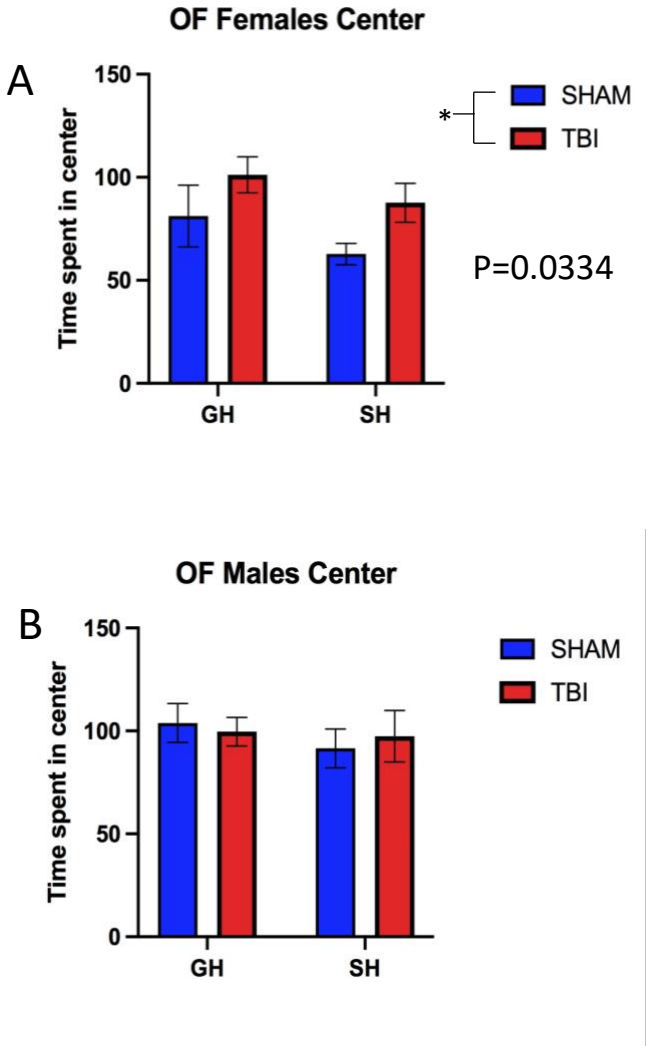


Figure 13: prior to the TORM test, the first day of the open field habituation was recorded and analyzed using EthoVision for the total time spent in center of the arena. Figure 13 A has a significant TBI interaction with a p value of 0.0334. There are no significant interactions in figure 13 B.

Shortly following the mild TBI, females showed a significant TBI effect, depicted by figure 13 A. TBI females spent significantly more time near the center compared to the SHAM females for both the group housed and single housed groups. The p value associated with this interaction was 0.0334. In figure 13 B, there were no significant effects found for any of the male groups.

Open Field Test Long Term Effects

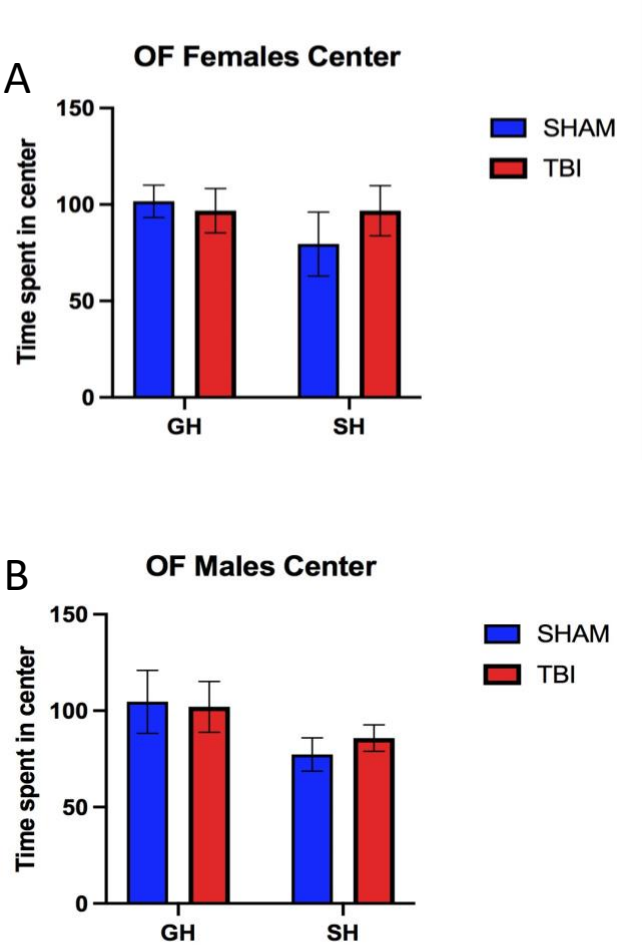
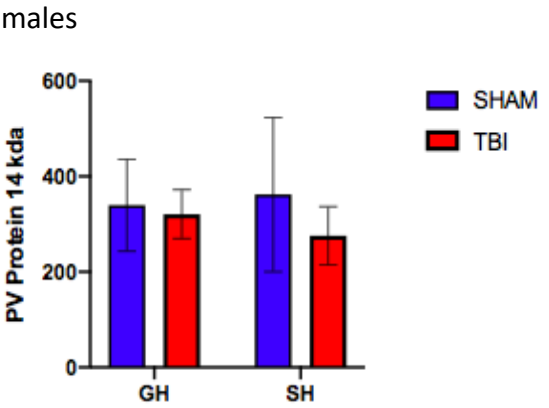


Figure 14: After reaching adulthood, the open field test was analyzed for total time spent in the center of the arena. There were no significant effects found across all groups for both females (figure 14 A) and males (figure 14 B).

There were no long-term effects associated with the open field test as depicted by the figures above. No significant interactions were found in the female groups (figure 14A). Similarly, to the females, there were no significant effects found for the males in the long term for the open field test (figure 14B).

Western Blot Parvalbumin Short Term Effects

A



B

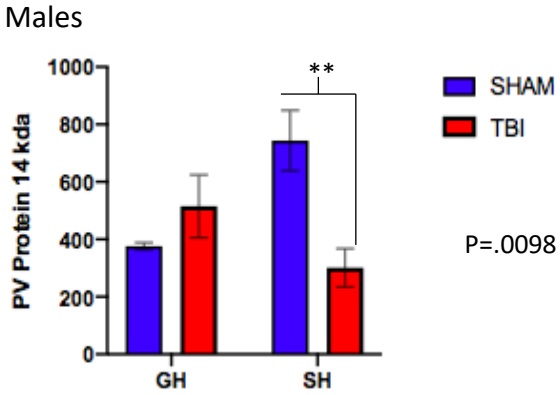


Figure 15: In figure A, there are no significant effects found in females. In figure B, there is a significant TBI effect found in the single housed males. SHAM single housed males have significantly higher Parvalbumin signal compared to TBI single housed males with a p value of 0.0098.

Shortly following the TBI, the PFC was dissected and analyzed for Parvalbumin signals using the Western Blot protocol described in the methods section. As depicted by the figures above, there were no significant effects found for the signal of Parvalbumin protein in females for the short-term effects of the TBI. However, there was a significant TBI effect in the single housed groups for the males. In the short term, SHAM single housed males showed higher Parvalbumin signals compared to TBI single housed males. The p value for this interaction was found to be 0.0098.

Western Blot Myelin Basic Protein Short Term Effects

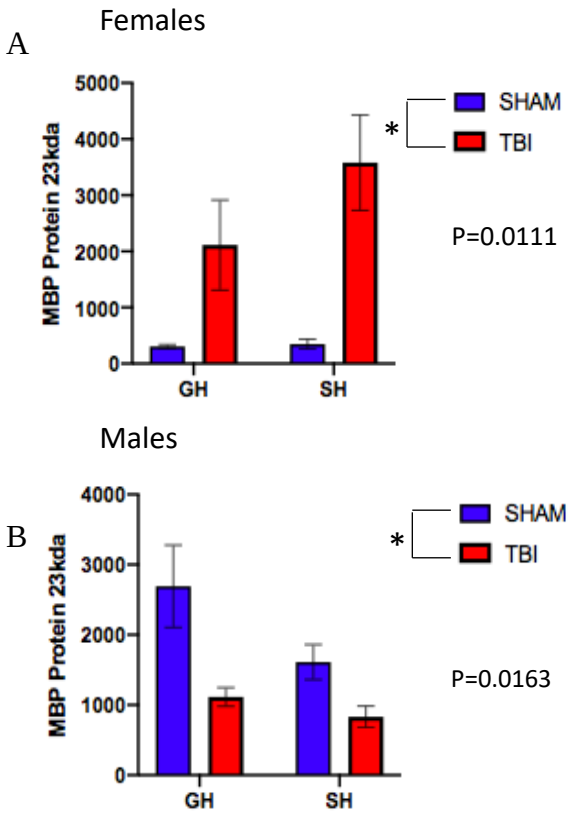
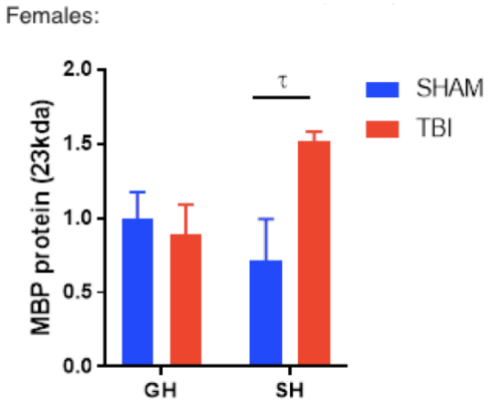


Figure 16: In figure A, there is a significant TBI effect for both single and group housed females. Females who experienced a TBI showed significantly higher signal for MBP compared to the SHAM females with a p value of 0.0111. In figure 16 B, there is a significant TBI effect with a p value of 0.0163. For the males, in figure 16 B, the SHAM groups have significantly higher signals for MBP compared to the TBI males.

To test for the short-term effects a TBI has on MBP expression, the PFC was dissected and was analyzed for MBP signals using the Western Blot procedure described earlier. In figure 16 A, there is a significant TBI effect showing that TBI females show significantly higher MBP signals compared to SHAM females for both single and group housed females. The p value for this interaction was found to be 0.0111. In figure 16 B, the opposite effect was found. SHAM males showed significantly higher signals for MBP compared to the TBI males for both housing conditions. The p value associated with this effect was found to be 0.0163.

Western Blot Myelin Basic Protein Long Term Effects

A



B

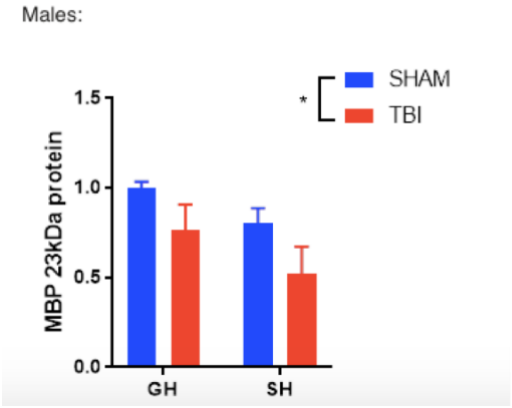


Figure 17: In figure A, there were no significant effects found for MBP signal across all groups. In figure 17 B, there was a significant TBI effect for both housing groups. SHAM males showed significantly higher MBP signals compared to the TBI males.

To test for the long-term effects a mild TBI has on the expression of myelin basic protein, the PFC was dissected after the mice reached adulthood and the Western Blot procedure was performed to measure the signals of MBP on the PFC. There were no significant effects found for MBP in the long term for any of the female groups (figure 17 A). However, for the males, there was a significant effect displaying that in the long-term, SHAM males display significantly higher signals for MBP compared to the TBI males.

Discussion

The present data involving a traumatic brain injury and social isolation in a mouse model shows that a TBI experienced during the critical development period of adolescence is associated with short-lasting effects such as difficulties in problem solving tasks and this effect is more profound in males. In the long term, there were no significant behavioral results for a TBI condition effect which shows that the mice were able to recover and did not experience long lasting difficulties with their executive functions due to the mild TBI.

In a recent study completed by Shishido et al. in 2018, they found that shortly following a TBI (7 days), the wild type mice showed a significantly lower spatial learning ability compared to SHAM wild type mice. However, 28 days following the TBI, they found that the cognitive impairment recovered 28 days following the TBI (Shishido et al., 2018) This effect was similar to the one found in our experiment. Shortly following the TBI, there was a significant effect depicting that TBI single housed males were significantly more latent to enter the goal box in trials associated with problem solving during the puzzle box test compared to SHAM single housed males, showing a significant TBI effect. However, after reaching adulthood, there was no significant effect found in the trials associated with problem solving for TBI condition thus proving that the mice recovered from their mild TBI suffered during adolescence.

Significant housing condition effects were found in both male and females in the long-term. Males and females were more latent to enter the goal box during the puzzle box assay for the trials associated problem solving. However, males also displayed significant longer times to enter the goal box in the long-term with trials associated with the trials assessing long term memory while females did not display these long-term effects.

In a study that explored the effects of social isolation, Guo et al. found that social isolation can in fact induce psychological behavioral changes (Guo et al., 2004). Not only did they determine that social isolation has an effect on behavior, they also noted that there were sex differences found within the socially isolated mice. They found that the male mice were more susceptible experience behavioral hindrances due to social isolation (Guo et al., 2004). In accordance with the study conducted by Guo et al, in a recent study completed by the Coutellier Lab, they also found that males were more susceptible to social stress (Page and Coutellier, 2018). The data presented in this experiment highlights findings similar to those described by Guo et al, and Page and Coutellier. Males showed not only a significant isolation effect in the puzzle box trials assessing long term memory, but they also experienced isolation effects in trials associated with long term memory. Females, on the other hand, only experienced isolation effects in the trials associated with problem solving and did not experience any significant isolation effects in the trials testing for long term memory.

We found a significant effect for females and their time spent in the center of the open field test. Shortly following the TBI surgery, TBI females spent significantly more time in the center compared to SHAM females ($p=0.0334$). No other significant effects were found for females in the open field test after reaching adulthood and no significant effects were found for males both in the short-term and long-term effects regarding the open field test.

Humans who have suffered a traumatic brain injury have a greater that 60% chance of developing psychiatric disorders such as anxiety and depression in the years following a TBI (brainline.org). A pilot study exploring the effects a mild TBI has on rodents showed that anxiety-like alterations occur in approximately 23% of mild TBI cases (Singh et al., 2016). The statistic from the pilot study might explain why there were no anxiety-like effects in males but

does not explain why females were less anxious since they spent more time in the center compared to the walls in the open field test. This anti-anxiolytic effect found in females following a mild TBI should be further explored.

The results from the Western Blot procedure which measured the concentrations of myelin basic protein (MBP) provide valuable insight to possible recovery mechanisms. In the short-term effects arising from a TBI, females that underwent a TBI surgery displayed a significant effect ($p=0.0111$) showing that TBI females have higher concentrations of MBP in comparison to their SHAM counterparts. This effect was opposite in the males for the short term. In the male groups, we found a significant effect between SHAM groups and TBI groups for MBP concentration. SHAM males had a significantly higher signal for MBP compared to the TBI groups—the opposite effect found in females. This same effect was found in the males for the long-term where SHAM males displayed a significantly higher MBP signal compared to the TBI males. There were no significant effects for MBP found in females in the long-term effects of a mild TBI.

According to ScienceDirect, myelin basic protein is a, "hydrophilic protein that plays a critical role in organization of myelin sheaths in both oligodendrocytes and Schwann cells." (sciencedirect.com). Myelin basic protein is also essential for normal myelination and axonal signal conduction (Su et al, 2012). Although the TBI used in this experiment was only mild, in a recent study conducted on pediatric patients and severe TBIs showed that there is an increase in MBP concentration in the cerebrospinal fluid after a severe TBI compared to the controls (Su et al, 2012). This might help explain why TBI females showed a significant effect of higher signaling for MBP compared to the SHAM females. The female results of MBP in the short term can provide valuable insight for possible TBI recovery mechanisms.

To help explain the why SHAM groups had higher signaling for MBP in the long term, a study conducted by Niu et al, provides valuable insight as to a mechanism by which MBP is degraded after experiencing a TBI. Traumatic brain injuries can lead to the over activation of calpain which can lead to the degradation of various target proteins (including MBP) and this degradation can eventually lead to neuronal death (Niu et al, 2020). In this same study, it was found that MBP levels were significantly decreased in traumatic brain tissues (Niu et al, 2020). The findings from the study conducted by Niu et al, can explain why there was a significant decrease in MBP signaling for TBI males in the short term and long term. However, the effect found for females in the short term should be explored further.

There were no significant results shown for females and parvalbumin signals but there was a significant effect of parvalbumin found in males. In the short term, SHAM single housed males had significantly higher parvalbumin signals in comparison to the TBI single housed males. This effect portrays that there is a TBI effect found for parvalbumin signaling in the short term for males. These results align with (Nichols et al., 2018) where they performed a TBI on rodents and analyzed PV expression in the injury sight. Similarly, to what we found regarding the higher signals for PV in SHAM males, they found that the loss of PV expression induced by a TBI was significantly greater nearer to the injury site.

Conclusion

Mild traumatic brain injury experienced during adolescence and then followed by social stress does not seem to significantly affect cognitive function in adulthood. Some short-term effects of a mild traumatic brain injury found in this study are more profound compared to the long-term effects, which show how recovery of a mild TBI is an explanation as to why there were no long term TBI cognitive effects. Social isolation does in fact lead to some cognitive deficits in adulthood, as shown by the housing effect found in females and males during the puzzle box test after given the opportunity to reach adulthood. It is important to note that the TBI administered in this study was mild, and administering a more severe TBI may provide more insight into the long-lasting effects of TBI experienced during adolescence.

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